In vitro digestion and domestic cooking improved the total antioxidant activity and carbohydrate-digestive enzymes inhibitory potential of selected edible mushrooms

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Abstract This study aimed to investigate the effect of in vitro digestion on the antioxidant activity and carbohydrate-digestive enzymes inhibitory potential of five edible mushrooms after subjected to four domestic cooking; namely, boiling, microwaving, steaming and pressure-cooking. The water extracts of raw (uncooked), cooked and in vitro digested mushrooms were compared for their water-soluble phenolic content (WPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), radical scavenging activity (TEAC and DPPH), anti- α -amylase and anti- α -glucosidase activities. Among the raw samples, *Lentinula edodes* possessed the highest antioxidant activities (FRAP, TEAC, DPPH) and WPC while *Pleurotus sajor-caju* displayed the highest TFC, anti- α -amylase and anti- α -glucosidase activities. The antioxidant and carbohydrate-digestive enzyme inhibitory activities significantly varied according to mushroom species and cooking methods applied. Short duration of microwaving (*Agaricus bisporus* and *Flammulina velutipes*), boiling (*Auricularia polytricha*) and pressure cooking (*L. edodes* and *P. sajor-caju*) yielded the best antioxidant and carbohydrate-digestive enzymes inhibition values in the mushroom extracts. TFC was positively correlated with the antioxidant activities and anti- α -glucosidase activity in the mushroom extracts. In vitro digestion significantly improved the total antioxidant and anti- α -glucosidase

activities but decreased the anti- α -amylase activity in the cooked mushroom extracts. Principle component analysis showed that in vitro digestion and the cooking process accounted for respective 48.9% and 19.7% of variation in the observed activities. Domestic cooking and in vitro digestion could potentiate the total antioxidant and carbohydrate-digestive enzymes inhibitory activities in the selected water extract of edible mushrooms.

Keywords Antioxidant · Carbohydrate-digestive enzymes · Cooking · Phenolic · In vitro digestion · Mushroom

Introduction

Mushrooms have been described as healthy foods due to their low calories, cholesterol, fat, and sodium content but rich in protein, carbohydrate, fibre, minerals and vitamins (Aida et al. 2009). Among the 2000 edible mushrooms, 35 species have gained much popularity worldwide due to their unique textures and aroma. Button mushroom (*Agaricus bisporus*) is the most widely cultivated species, followed by shiitake (*Lentinula edodes*), oyster (*Pleurotus* spp.), jelly ear (*Auricularia* spp.) and golden needle (*Flammulina velutipe*) (Aida et al. 2009). *A. bisporus* is well known to the Western countries since they are commonly found in Europe and North America. In contrast, *Auricularia* spp., *L. edodes* and *F. velutipe* are mostly cultivated in South Pacific and East Asian countries such as India, China, Taiwan, Singapore, Japan, Korea and Thailand (Ghorai et al. 2009).

In Asia, mushrooms are recognized as functional foods as they contain a variety of biological active compounds with therapeutic potential such as alkaloids, β -glucan,

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lectins, peptides, phenolics, sterols (ergosterols) and terpenes (Bach et al. 2017). The synergistic effect exerted by these compounds in the mushrooms could alleviate the development of many inflammatory diseases associated with oxidative stress such as type 2 diabetes mellitus (De Silva et al. 2012). For instance, a water-soluble polysaccharide-peptide complex from the fruiting body of *Pleurotus* spp. displayed significantly high antioxidant and hypoglycemic activities (Li et al. 2012).

Generally, mushrooms are either consumed raw as salad or cooked by microwaving, stir-frying, pressure-cooking, steaming or boiling. Thermal processing such as domestic cooking has been shown to cause differential effect on the phytochemicals in plant foods (Ng et al. 2011, 2014; Ribas-Agustí et al. 2017). The phytochemicals present in the mushrooms, particularly the antioxidants varied according to mushroom species, cell wall matrix, and the properties of phytochemicals such as their thermal stability (Ng and Tan 2017). More importantly, the bioaccessibility of phytochemicals in foods could be altered by drastic process such as gastrointestinal digestion due to the effect of different pH, enzymatic polymerization, and molecule hydrophobicity (Siracusa et al. 2011). Hence, it is essential to quantify the proportion of phytochemicals and their bioactivity available for absorption into the biological system after the digestion. This is especially important for mushrooms with significant amount of indigestible polysaccharides that might affect the extractability of phytochemicals (Soler-Rivas et al. 2009).

Although the nutritional profile of different edible mushrooms have been extensively studied, only a relatively small number of reports on the changes of antioxidant and carbohydrate-digestive enzymes inhibition activities in the cooked and in vitro digested mushrooms are available but limited to a few selected cultivars (Heleno et al. 2015; Soler-Rivas et al. 2009). The present study is aimed to address this by investigating the water soluble phenolic and flavonoid content, total antioxidant activity and carbohydrate-digestive enzymes inhibition potential of five edible mushrooms; namely, *A. bisporus*, *A. polytricha*, *F. velutipes*, *L. edodes* and *P. sajor-caju* after subjecting to four types of domestic cooking and followed by in vitro digestion. This study utilizes an in vitro digestion model which mimics human gastrointestinal digestive process to evaluate the digestive stability of phenolic contents on their reducing power and radical scavenging activities in the digested mushroom products.

Materials and methods

Chemicals

All chemicals used in the study were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin), 2,4,6-tripyridyl-s-triazine (TPTZ), 3,4,5-trihydroxybenzoic acid (gallic acid), 3,5-dinitrosalicylic acid (DNS), 4-nitrophenyl- α -D-glucopyranoside (PNPG), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), α -amylase (human saliva type IX-A, EC 3.2.1.1), α -amylase (porcine pancreatic Type IV-B, EC 3.2.1.1), α -glucosidase (*Saccharomyces cerevisiae*, EC 3.2.1.20), aluminium chloride (AlCl₃), bile extract porcine, calcium chloride (CaCl₂), iron(II) sulphate heptahydrate (FeSO₄·7H₂O), L-ascorbic acid (C₆H₈O₆), pancreatin from porcine pancreas, pepsin from porcine gastric mucosa, potassium acetate (C₂H₃KO₂), potassium chloride (KCl), sodium acetate trihydrate (C₂H₃NaO₂·3H₂O), starch and voglibose, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Absolute ethanol (CH₃CH₂OH), Folin-Ciocalteu's reagent, fuming hydrochloric acid (HCl), glacial acetic acid (C₂H₄O₂), iron(III) chloride hexahydrate (FeCl₃·6H₂O), potassium peroxodisulphate (K₂O₈S₂), sodium carbonate (Na₂CO₃), sodium hydroxide (NaOH) and sodium phosphate (C₂H₆O) were purchased from Merck (Darmstadt, Germany). 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Roche (Baden-Württemberg, Germany). Ammonium carbonate (NH₄)₂CO₃, magnesium chloride hexahydrate (MgCl₂·6H₂O), potassium dihydrogen phosphate (KH₂PO₄), sodium chloride (NaCl) and sodium bicarbonate (NaHCO₃) and were purchased from Fisher Scientific (Loughborough, UK).

Equipments

800 W-microwave oven (Sanyo Electric Co., Ltd, Osaka, Japan), autoclave and sterilizer (Hirayama Manufacturing Corp., Saitama, Japan), centrifuge (Eppendorf AG, Hamburg, Germany), Epoch microplate spectrophotometer (Biotek Instrument Inc., Vermont, USA), electrical blender (Panasonic Corporation., Osaka, Japan), hot air oven (Memmert GmbH & Co. KG, Schwabach, Germany), hot plate stirrer (Corning Inc., New York, USA), stainless steel steam rack plate 26 cm (Saten Stainless Steel Public Co. Ltd., Pravat, Thailand), vortex mixer (IKA-Werke GmbH & Co. KG, Staufen, Germany), water bath (Memmert GmbH & Co. KG, Schwabach, Germany).

Sample collection

Five commercially-cultivated fresh mushroom species; namely, *Agaricus bisporus* (white button), *Auricularia polytricha* (black jelly), *Flammulina velutipes* (enokitake), *Lentinula edodes* (shiitake) and *Pleurotus sajor-caju* (grey oyster) were purchased from a local wholesale market in Semenyih, Malaysia. The selected mushrooms comprised of complete fruiting bodies and morphologically identified according to Huang (1998). Each mushroom sample (biological triplicates) was washed with distilled water and blot-dried. The edible portions were sliced into small pieces (approximate 1–2 cm³) and divided into 100 g portions for each cooking regime.

Moisture content

The moisture content of each mushroom sample was measured based on AOAC official gravimetric method 934.06 (AOAC International 2006).

Cooking and juice extraction

The cooking procedure was adapted from Ng et al. (2011) to mimic the domestic cooking styles. For pressure-cooking, each mushroom sample was autoclaved with 100 mL of distilled water at 121 °C under 2 MPa for 20 min; for steam cooking, the mushroom samples were cooked with steam on a steamer rack plate over 95 °C water in a closed water bath for 10 min; for microwave cooking, the mushroom samples were microwaved in 100 mL of distilled water with a 800 W microwave oven at high power for 10 min; for boiling, each mushroom sample was boiled in 100 mL of boiling distilled water on a hot plate at 100 °C for 10 min. The glass beakers were loosely covered to prevent evaporation during the cooking. All cooked mushroom samples and the cooking water were cooled rapidly on ice and homogenised with an electrical blender. For raw samples, one hundred grams of each mushroom (biological triplicates) were homogenised with 100 mL of distilled water. The homogenate were filtered and centrifuged at 3220×g for 20 min at 4 °C to obtain a clear supernatant. Five millilitre of each supernatant was subjected to in vitro digestion immediately while the remaining extracts were stored at –20 °C for bioactivity analyses.

In vitro digestion

The in vitro digestion was performed according to the method by Minekus et al. (2014). In brief, five millilitre of simulated salivary fluid was mixed with equivalent volume of each mushroom extract (1 g/mL) cooked with different

methods. Distilled water was used to replace the cooked mushroom extracts as digestion negative control. After the addition of 1500 U/mL α -amylase (human saliva type IX-A), the solution was adjusted to pH 7 and incubated for 2 min at 37 °C to complete the oral digestion phase. Ten millilitre of simulated gastric fluid with 25,000 U/mL pepsin was then added into the solution and adjusted to pH 3 with 1 M hydrochloric acid. The solution was incubated for 2 h at 37 °C in a shaking water bath (200 rpm) to complete the gastric digestion phase. The intestinal digestion phase was started with the addition of twenty millilitre of simulated duodenal fluid containing 800 U/mL porcine pancreatin and 160 mM bile, followed by the adjustment of pH to 7 with 1 M sodium hydroxide. The solution was then incubated for another 2 h at 37 °C in a shaking water bath (200 rpm). Lastly, the digested extracts were centrifuged at 3220×g for 15 min at 4 °C and the supernatants were stored at –20 °C in dark. All sample extracts were analysed with three technical replicates in each of the bioassay within 2 weeks.

Ferric reducing antioxidant power (FRAP)

The ferric reducing power of the mushroom extracts (raw, cooked and digested) was measured according to Benzie and Strain (1996). FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in 40 mM HCl) and FeCl₃·6H₂O (20 mM) in the ratio of 10:1:1. Ten μ L of each mushroom extract was incubated with 300 μ L of FRAP reagent for 4 min at room temperature prior to the measurement of absorbance at 593 nm with a microplate spectrophotometer. Iron(III) sulphate (0–1000 μ M) was used as the standard. The FRAP value was expressed as millimole iron(II) equivalent per 100 g fresh weight sample (mmol Fe²⁺/100 g FW).

Trolox equivalent antioxidant capacity (TEAC)

The ABTS radical scavenging activity in the mushroom extracts (raw, cooked and digested) was measured according to Re et al. (1999). ABTS radicals were generated by incubating ABTS diammonium salt (7 mM) with potassium persulfate solution (104 mM) for 12 to 16 h. The ABTS radical solution was diluted in ethanol to prepare a working ABTS reagent with an absorbance of 0.7 ± 0.02 at 734 nm. Ten μ L of each mushroom extract was incubated with 100 μ L of working ABTS reagent for 1 min at room temperature prior to the measurement of absorbance at 734 nm with a microplate spectrophotometer. Trolox (0–250 μ g/mL) was used as the standard. The TEAC value was expressed as millimole trolox equivalent per 100 g fresh weight sample (mmol trolox/100 g FW).

DPPH radical scavenging capacity

The DPPH radical scavenging activity in the mushroom extracts (raw, cooked and digested) was measured according to Gerhauser et al. (2003). Briefly, 5 μ L of each mushroom extract was incubated with 195 μ L of DPPH reagent (100 μ M in ethanol) at room temperature in dark. The change in the absorbance of the reaction mixture was monitored for 120 min, with 15 min interval, at 515 nm with a microplate spectrophotometer. Ascorbic acid (0–1000 μ M) with half maximal inhibitory concentration (IC_{50}) of 22.16 μ M was used as the standard. The DPPH radical scavenging capacity was expressed as millimole ascorbic acid equivalent per 100 g fresh weight sample (mmol AA/100 g FW).

Water-soluble phenolic content (WPC)

The WPC in the mushroom extracts (raw, cooked and digested) was measured according to Singleton and Rossi (1965). Fifty μ L of each mushroom extract was mixed with equal volume of Folin-Ciocalteu reagent (10% v/v) and incubated for 3 min at room temperature in dark, followed by the addition of 100 μ L of Na_2CO_3 (10% w/v). The blue colour in the reaction was allowed to develop for 60 min prior to the measurement of absorbance at 750 nm with a microplate spectrophotometer. Gallic acid (0–100 μ g/ml) was used as the standard. The WPC value was expressed as milligrams gallic acid equivalent per 100 g fresh weight sample (mg GAE/100 g FW).

Total flavonoid content (TFC)

The TFC in the mushroom extracts (raw, cooked and digested) was measured according to Chang et al. (2002). Twenty five μ L of each mushroom extract was mixed with 75 μ L ethanol, 10 μ L $C_2H_3KO_2$ (1 M), 10 μ L $AlCl_3$ (10% w/v) and 140 μ L distilled water. After 30-min incubation at room temperature in dark, the absorbance of the reaction mixtures was measured at 415 nm with a microplate spectrophotometer. Sample blank was prepared by substituting the $AlCl_3$ with the same amount of distilled water. Quercetin (0–1000 μ g/ml) was used as the standard. The TFC value was expressed as milligrams quercetin equivalent per 100 g fresh weight sample (mg QE/100 g FW).

Anti- α -amylase activity

The anti- α -amylase activity of the mushroom extracts was measured according to Telagari and Hullatti (2015). Twenty μ L of each mushroom extract was pre-incubated with 50 μ L sodium phosphate buffer (100 mM, pH 6.8) and 10 μ L α -amylase (2 U/mL) at 37 °C for 20 min,

followed by the addition of 20 μ L soluble starch (1% w/v) in sodium phosphate buffer. After 30-min incubation at 37 °C, 100 μ L DNS reagent was then added and incubated for another 10 min at 95 °C. The absorbance of the reaction mixture was measured at 540 nm with a microplate spectrophotometer. Reagent blank (reaction without α -amylase) was used to exclude the background absorbance. Voglibose (0–40 mg/ml) with half maximal inhibitory concentration (IC_{50}) of 19.33 mg/ml was used as the standard. The anti- α -amylase activity was expressed as percentage inhibition (%) as follows:

$$\text{Anti-}\alpha\text{-amylase activity (\%)} \\ = (1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{blank}}) \times 100$$

where Abs and blank refer to the absorbance and reagent blank respectively.

Anti- α -glucosidase activity

The anti- α -glucosidase activity of the mushroom extracts was measured according to Telagari and Hullatti (2015). Twenty μ L of each mushroom extract was pre-incubated with 50 μ L sodium phosphate buffer (100 mM, pH 6.8) and 10 μ L α -glucosidase (1 U/mL) for 15 min at 37 °C. Following the addition of 20 μ L PNPG substrate (5 mM), the reaction mixture was incubated for another 20 min at 37 °C prior to adding 50 μ L Na_2CO_3 (0.1 M) to terminate the reaction. The absorbance of the reaction mixture was measured at 405 nm with a microplate spectrophotometer. Reagent blank (reaction without α -glucosidase) was used to exclude the background absorbance. Voglibose (0–40 mg/ml) with half maximal inhibitory concentration (IC_{50}) of 10.47 mg/ml was used as the standard. The anti- α -glucosidase activity was expressed as percentage inhibition (%) as follows:

$$\text{Anti-}\alpha\text{-glucosidase activity (\%)} \\ = (1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{blank}}) \times 100$$

where Abs and blank refer to the absorbance and reagent blank respectively.

Antioxidant index (AI)

The AI of each mushroom extract was calculated as an average relative percentage value obtained through the five different antioxidant assays; namely, FRAP, DPPH, TEAC, WPC and TFC. It was used to relatively rank the mushroom sample extracts based on their overall antioxidant capacity according to Puttaraju et al. (2006). The highest value in each assay was considered as 100 while the remaining lower values were converted based on the numerical scale. The mushroom sample extracts were

classified into high (76–100%), moderate (50–75%), low (25–49%), and very low (0–24%) AI species.

Statistical analysis

All data were expressed as mean of nine independent determinations $\pm$ standard deviation (SD). Statistical mean difference among the mushroom extracts was determined by using analysis of variance (ANOVA) with Tukey post hoc test. Pearson's linear correlation and regression analyses were used to determine the association between the investigated activity parameters. Principle component analysis was used to determine the influence of cooking and digestion on the investigated variables in the whole data set. A p value of less than 0.05 was considered statistically significant. All data analysis was performed using GraphPad Prism 6 software (GraphPad Software, USA) and XLSTAT 2014 software for Microsoft Excel program (Addinsoft Inc., New York, NY, USA).

Results and discussion

The moisture content, antioxidant and carbohydrate digestive enzyme inhibitory activities in raw mushrooms

All the five selected edible mushrooms in this study belonged to different genera. Their moisture content were significantly ($p < 0.05$) different from each other, ranging from 83.1 to 94.8%. *A. bisporus* showed the highest moisture content ($94.8 \pm 0.01\%$), followed by *P. sajor-caju* ($92.5 \pm 0.3\%$), *F. velutipes* ($89.8 \pm 0.1\%$), *A. polytricha* ($84.9 \pm 0.1\%$) and *L. edodes* ($83.1 \pm 0.2\%$).

The antioxidant activities in the mushroom extracts were estimated based on their ferric reducing power (FRAP) as well as radical scavenging potential (TEAC and DPPH) (Table 1). *L. edodes* showed the highest FRAP value, followed by *A. bisporus*, *F. velutipes*, *A. polytricha* and *P. sajor-caju*. The standard ascorbic acid used in DPPH radical scavenging assay scavenged 50% DPPH radicals at $22.16 \mu\text{M}$ as compared to $21.6 \mu\text{M}$ (Ng et al. 2011) shown in our previous study. *L. edodes* was the most effective radical scavengers among the raw mushrooms by showing the highest ABTS and DPPH radical scavenging activities. The TEAC values of *A. bisporus*, *F. velutipes* and *P. sajor-caju* were comparable while similar finding was found in the DPPH radical scavenging activities of *A. polytricha*, *A. bisporus* and *F. velutipes*. The WPC of raw mushroom extracts showed similar trend when compared to their antioxidant activities (Table 1). The decreasing WPC of raw mushrooms were in the following order: *L. edodes* > *A. bisporus* > *F. velutipes* > *P. sajor-caju* > *A.*

polytricha. On the contrary, *P. sajor-caju* displayed the highest TFC, followed by *A. bisporus*, *F. velutipes*, *L. edodes* and *A. polytricha*. The variation of WPC and TFC among the mushrooms suggests the presence of aglycone phenolics and their glycosides with different water-extraction efficiency. In addition, flavonoids were the major phenolic compounds for *Pleurotus* spp., particularly catechin, quercetin and chrysin (Mohamed and Farghaly 2014). This corresponds with the current finding that *P. sajor-caju* possessed the highest TFC among the five selected mushrooms. In tandem, FRAP, TEAC, WPC, and TFC values in the raw *A. bisporus*, *F. velutipes* and *L. edodes* samples were comparable to the values shown in previous studies (Gan et al. 2013; Ng and Tan 2017).

The management of diabetes mellitus is associated with the control of postprandial hyperglycemia. The two key rate-limiting carbohydrate-digestive enzymes are pancreatic α -amylase and intestinal α -glucosidase (Telagari and Hullatti 2015). The inhibition of these enzymes can delay the digestion of polysaccharide in human intestine and thus reduce the postprandial blood sugar. In this study, the half maximal inhibitory concentration (IC_{50}) of positive control, voglibose used in the anti- α -amylase and anti- α -glucosidase assays were comparable to a previous finding (Telagari and Hullatti 2015). The anti- α -amylase activities (26.5–76.4%) in the mushroom extracts were generally stronger than their anti- α -glucosidase activities (13.4–39.7%) (Table 1) but weaker than the positive control. Interestingly, *P. sajor-caju* with the highest TFC also showed the highest anti- α -amylase and anti- α -glucosidase activities. Comparable anti- α -amylase and anti- α -glucosidase activities were found in *L. edodes*, *A. bisporus* and *F. velutipes* extracts while *A. polytricha* possessed the lowest activities.

Based on the AI value shown in Table 2, the raw edible mushroom samples were grouped into three categories: high, moderate and very low AI species. *L. edodes* was ranked as high AI mushroom species due to its consistent strong activity trend shown across the 4 antioxidant assays (FRAP, TEAC, DPPH and WPC). This was in agreement with Cheung et al. (2003) which reported high phenolic and radical scavenging activity in the water extract of *L. edodes*. Three mushrooms with AI between 51 and 66; namely, *A. bisporus*, *F. velutipes* and *P. sajor-caju*, were classified as moderate AI mushroom species. *A. polytricha* with its relatively low activity trend in three antioxidant assays, was labelled as very low AI mushroom species. When combined with other therapeutic properties found in the mushrooms such as their carbohydrate-digestive enzymes inhibition values, the AI could be an alternative approach to rank the potential health benefit of mushrooms.

Table 1 The total antioxidant activity and carbohydrate-digestive enzyme inhibition potential of raw (uncooked) mushroom samples

Raw (uncooked) mushrooms	Antioxidant activity			Phenolic content		Carbohydrate digestive enzyme inhibitory activity	
	FRAP (mmol Fe ²⁺ /100 g)	TEAC (mmol trolox/100 g)	DPPH ^c (mmol AA/100 g)	WPC (mg GAE/100 g)	TFC (mg QE/100 g)	Anti- α -amylase (% inhibition)	Anti- α -glucosidase (% inhibition)
<i>A. bisporus</i>	0.16 $\pm$ 0.02 ^a	0.08 $\pm$ 0.00 ^a	0.06 $\pm$ 0.00 ^a	26.33 $\pm$ 0.70 ^a	78.67 $\pm$ 8.80 ^a	30.58 $\pm$ 9.64 ^a	17.64 $\pm$ 1.33 ^a
<i>A. polytricha</i>	0.02 $\pm$ 0.01 ^b	0.01 $\pm$ 0.00 ^b	0.05 $\pm$ 0.00 ^{ac}	6.68 $\pm$ 0.07 ^b	18.78 $\pm$ 5.18 ^b	26.53 $\pm$ 3.30 ^a	13.35 $\pm$ 1.87 ^b
<i>F. velutipes</i>	0.12 $\pm$ 0.00 ^a	0.08 $\pm$ 0.01 ^{ac}	0.05 $\pm$ 0.00 ^{ac}	24.27 $\pm$ 0.10 ^a	41.44 $\pm$ 10.02 ^c	29.18 $\pm$ 2.18 ^a	22.64 $\pm$ 1.56 ^c
<i>L. edodes</i>	0.33 $\pm$ 0.02 ^c	0.11 $\pm$ 0.01 ^a	0.09 $\pm$ 0.00 ^b	36.44 $\pm$ 0.04 ^c	31.89 $\pm$ 7.29 ^c	33.49 $\pm$ 5.84 ^a	21.39 $\pm$ 1.56 ^c
<i>P. sajor-caju</i>	0.02 $\pm$ 0.00 ^b	0.06 $\pm$ 0.00 ^c	0.04 $\pm$ 0.01 ^c	14.72 $\pm$ 0.58 ^d	112.00 $\pm$ 5.51 ^d	76.38 $\pm$ 1.03 ^b	39.66 $\pm$ 2.08 ^d

Values represent mean $\pm$ standard deviations (SD) of nine independent determinations. Values within the same column followed by different superscript lower case letters (a, b, c, d) are significantly different at $p < 0.05$

^cThe half maximal inhibitory concentration (IC₅₀) for ascorbic acid standard was 22.16 μ M

Table 2 The antioxidant index (AI) of five raw (uncooked) mushroom samples

Raw sample	FRAP (mmol Fe ²⁺ /100 g)	WPC (mg GAE/100 g)	TFC (mg QE/100 g)	TEAC (mmol trolox/100 g)	DPPH ^a (mmol AA/100 g)	Relative % FRAP	Relative % WPC	Relative % TFC	Relative % TEAC	Relative % DPPH	AI ^a	Category ^b
<i>L. edodes</i>	0.33	36.44	31.89	0.11	0.09	100	100	29	100	100	86	High
<i>A. bisporus</i>	0.16	26.33	78.67	0.08	0.06	49	72	70	73	67	66	Moderate
<i>F. velutipes</i>	0.12	24.27	41.44	0.08	0.05	36	67	37	73	56	54	Moderate
<i>P. sajor-caju</i>	0.02	14.72	112.00	0.06	0.04	6	40	100	55	56	51	Moderate
<i>A. polytricha</i>	0.02	6.68	18.78	0.01	0.05	6	18	17	9	56	21	Very low

Relative percentage value calculated based on the highest value in each antioxidant assay

^aAverage value of the relative percentages from five antioxidant assays

^bAI species category: high (76–100%), moderate (50–75%), low (25–49%), and very low (0–24%)

Effect of domestic cooking on the antioxidant and carbohydrate-digestive enzyme inhibition activities in the mushrooms

Table 3 depicts the changes of antioxidant and carbohydrate-digestive enzyme inhibition activities in five edible mushrooms cooked with different methods. Regardless of cooking methods, the FRAP values of cooked *F. velutipes* and *L. edodes* samples were significantly ($p < 0.05$) decreased by at least 50% when compared to their raw counterparts. Pressure cooking with prolonged cooking time (20 min) exerted the strongest impact on *F. velutipes* (– 67%). However, boiling (150%) and steaming (350%) significantly ($p < 0.05$) increased the FRAP values of *A. polytricha*. Similar finding was found in the boiled (350%), microwaved (50%) and pressured cooked (200%) samples of *P. sajor-caju*. On the contrary, the FRAP value of *A. bisporus* was not affected after the cooking. The radical scavenging activities in the mushroom extracts were less affected by the cooking process when compared to their

FRAP values. Pressure cooking generally decreased the TEAC values of *A. bisporus* (– 75%) and *F. velutipes* (– 38%). Similar trend was observed in *L. edodes* cooked by steam and microwave (– 45%) as well as in *P. sajor-caju*'s boiled (– 33%) and microwaved (– 17%) extracts. However, boiling (400%), microwaving (400%) and pressure cooking (300%) increased the TEAC value of *A. polytricha*. All cooking methods significantly improved the DPPH radical scavenging activities of *F. velutipes* (160–200%), *L. edodes* (33–150%) and *P. sajor-caju* (120–160%). Tan et al. (2015) reported a decrease of DPPH radical scavenging activity in *Pleurotus* spp. after the cooking process. This conflicting result could be attributed to the different mushroom species and cooking duration used in their study. The improvement of antioxidant activity in the cooked mushroom extracts could be contributed by the leeching of active antioxidants from the fibrous complexes of mushroom matrix into the cooking water. On the contrary, boiling, microwaving and steaming significantly decreased the DPPH radical scavenging

Table 3 The total antioxidant activity and carbohydrate-digestive enzyme inhibition potential of cooked mushroom samples

Cooked mushrooms	Antioxidant activity				Phenolic content				Carbohydrate digestive enzyme inhibition potential			
	FRAP (mmol Fe ²⁺ /100 g)	Δ (%)	TEAC (mmol trolox/100 g)	Δ (%)	DPPH (mmol AA/100 g)	Δ (%)	WPC (mg GAE/100 g)	Δ (%)	TFC (mg QE/100 g)	Δ (%)	Anti-α-amylase (% inhibition)	Anti-α-glucosidase (% inhibition)
<i>A. bisporus</i>												
b	0.13 ± 0.00		0.11 ± 0.01		0.03 ± 0.00 ^b	– 50	7.32 ± 0.97 ^b	– 72	46.89 ± 1.25		7.95 ± 3.55 ^a	– 74 16.73 ± 2.10 ^a
m	0.11 ± 0.00		0.11 ± 0.00 ^a	38	0.01 ± 0.01 ^b	– 83	9.85 ± 0.18 ^d	– 63	43.39 ± 4.66		90.86 ± 5.91 ^a	197 19.42 ± 2.99
s	0.11 ± 0.00		0.11 ± 0.01		0.04 ± 0.01 ^a	– 33	8.09 ± 0.38 ^c	– 69	32.83 ± 4.51 ^b		55.81 ± 2.19	21.09 ± 3.65
p	0.11 ± 0.00		0.02 ± 0.01 ^b	– 75	0.04 ± 0.01		10.03 ± 0.11 ^c	– 62	59.11 ± 10.30 ^b		88.87 ± 0.45 ^a	191 14.67 ± 2.01
<i>A. polytricha</i>												
b	0.05 ± 0.00 ^a	150	0.05 ± 0.00 ^b	400	0.18 ± 0.01 ^d	260	4.76 ± 0.05 ^d	– 29	15.89 ± 7.32		9.43 ± 6.71	15.96 ± 0.52
m	0.02 ± 0.00		0.05 ± 0.01 ^b	400	0.16 ± 0.01 ^d	220	4.60 ± 0.03 ^d	– 31	23.56 ± 7.22		41.57 ± 6.48	10.55 ± 3.14
s	0.09 ± 0.00 ^b	350	0.05 ± 0.01		0.02 ± 0.00 ^a	– 60	6.60 ± 0.48		41.11 ± 4.63 ^b	119	43.29 ± 4.63	0.88 ± 0.58 ^a
p	0.01 ± 0.00		0.04 ± 0.01 ^b	300	0.17 ± 0.01 ^d	240	2.71 ± 0.09 ^d	– 59	16.78 ± 7.43		76.70 ± 3.40 ^b	189 10.11 ± 2.69
<i>F. velutipes</i>												
b	0.05 ± 0.00 ^b	– 58	0.06 ± 0.01		0.13 ± 0.01 ^b	160	7.08 ± 0.14 ^d	– 71	27.28 ± 3.13		34.07 ± 2.35 ^a	17 27.93 ± 3.46
m	0.06 ± 0.01 ^a	– 50	0.06 ± 0.00		0.14 ± 0.01 ^a	180	6.83 ± 0.16 ^d	– 72	31.06 ± 5.25		50.36 ± 1.19 ^b	73 15.13 ± 1.85
s	0.05 ± 0.00 ^c	– 58	0.05 ± 0.00		0.15 ± 0.00 ^d	200	8.37 ± 0.34 ^d	– 66	53.17 ± 8.59		42.64 ± 2.04 ^a	46 5.36 ± 1.12 ^b
p	0.04 ± 0.00 ^d	– 67	0.05 ± 0.00 ^a	– 38	0.14 ± 0.01 ^b	180	6.82 ± 0.20 ^d	– 72	47.00 ± 16.12		86.16 ± 2.84 ^b	195 11.62 ± 0.88 ^a
<i>L. edodes</i>												
b	0.12 ± 0.00 ^b	– 64	0.08 ± 0.01		0.08 ± 0.01	33	7.98 ± 0.62 ^d	– 78	49.89 ± 6.80		34.38 ± 6.43	16.73 ± 2.10
m	0.13 ± 0.00 ^b	– 61	0.06 ± 0.01 ^a	– 45	0.09 ± 0.00 ^c	50	8.15 ± 0.53 ^d	– 78	46.72 ± 12.61		50.64 ± 5.62	6.27 ± 1.62 ^a
s	0.12 ± 0.00 ^b	– 64	0.06 ± 0.01 ^b	– 45	0.08 ± 0.01	33	7.07 ± 0.21 ^d	– 81	70.61 ± 11.17		5.33 ± 2.78 ^a	4.41 ± 2.17 ^a
p	0.12 ± 0.00 ^b	– 64	0.07 ± 0.01		0.15 ± 0.01 ^a	150	5.39 ± 0.04 ^d	– 85	69.11 ± 2.55 ^a	117	67.98 ± 3.10 ^a	103 4.29 ± 1.78 ^b
<i>P. sajor-caju</i>												
b	0.09 ± 0.01 ^b	350	0.04 ± 0.00 ^a	– 33	0.12 ± 0.01 ^a	140	6.94 ± 0.22 ^b	– 53	57.11 ± 11.11 ^a	– 49	16.13 ± 3.70 ^b	– 79 3.44 ± 3.46 ^a
m	0.03 ± 0.00 ^a	50	0.05 ± 0.00 ^a	– 17	0.13 ± 0.00 ^b	160	9.46 ± 0.23 ^b	– 36	42.94 ± 5.23 ^a	– 62	61.41 ± 2.78 ^a	– 20 19.32 ± 1.75 ^a
s	0.03 ± 0.00		0.05 ± 0.01		0.12 ± 0.01 ^a	140	7.98 ± 0.63 ^a	– 46	38.78 ± 6.31 ^a	– 65	58.40 ± 1.91 ^c	– 24 24.14 ± 1.07 ^b
p	0.06 ± 0.00 ^b	200	0.06 ± 0.01		0.11 ± 0.01 ^b	120	8.86 ± 0.39 ^b	– 40	61.33 ± 12.09 ^a	– 45	67.28 ± 1.84 ^a	– 12 13.98 ± 2.01 ^a

The values are presented as mean ± standard deviations (SD) of nine independent determinations; Δ—Significant variation when compared to the respective raw (uncooked) sample; b—boiled samples; m—microwaved samples; p—pressure cooked samples; s—steamed samples; values within the same column followed by superscript lower case letters 'a' ($p < 0.05$), 'b' ($p < 0.01$), 'c' ($p < 0.005$) and 'd' ($p < 0.001$) were statistically different from their respective control species in Table 1

activity in *A. bisporus* (– 33% to – 50%) and *A. polytricha* (– 60%) extracts.

WPC in all mushroom extracts were significantly decreased by 29% to 85% after the cooking process. The decrease of WPC in all cooked mushroom extracts, particularly *F. velutipes* and *L. edodes*, were accompanied by the decrease of their FRAP values, suggesting the contribution of WPC to their antioxidant activity. However, this trend was absent in *A. bisporus*, *A. polytricha* and *P. sajor-caju*, possibly due to the presence of heat-resistant antioxidant compounds other than phenolic such as β -glucan and amino acid, ergothioneine (Soler-Rivas et al. 2009) as well as heat-stable superoxide dismutase (Cheng et al. 2012) and quinone oxidoreductase (Ng et al. 2014). Steaming and pressure-cooking improved the TFC in *A. polytricha* and *L. edodes* by 119% and 117% respectively but significant loss of TFC was observed in all the cooked *P. sajor-caju* extracts. Overall, cooking had lesser impact on the TFC in the mushroom extracts when compared to the WPC. This study hypothesizes that the thick and firm cell wall structure of mushrooms protected the flavonoids from degradation by cooking heat.

The anti- α -amylase activities in all cooked *P. sajor-caju* extracts were significantly reduced by 12% to 79%. However, opposite trend was found in *F. velutipes* extracts (17–195%). Interestingly, pressure-cooking significantly improved the anti- α -amylase activities of all mushrooms by as much as 103% to 195% except for *P. sajor-caju*. Cooking either decreased or caused no significant change in the anti- α -glucosidase activity in the mushrooms. Significant loss of anti- α -glucosidase activities were recorded in boiled *A. bisporus* (– 5%) and pressure-cooked samples of *F. velutipes* (– 49%) and *L. edodes* (– 80%). The decreasing effect of steam cooking on anti- α -glucosidase activity in the mushroom extracts was in the following order: *A. polytricha* (– 93%) > *L. edodes* (– 79%) > *F. velutipes* (– 76%) > *P. sajor-caju* (– 39%). All cooking methods significantly decreased the anti- α -glucosidase activity in *P. sajor-caju*, possibly due to the formation of phenol–protein complex with impaired activity (Jimenez-Monreal et al. 2009). The differential effects observed on the carbohydrate–digestive enzyme inhibition activity in the cooked mushroom extracts were likely due to the presence of phytochemicals with different thermal stability that influenced their binding with the carbohydrate-digestive enzymes (Silva et al. 2018). This study is in agreement with previous similar findings (Ng and Tan 2017; Sánchez 2017) that domestic cooking could either increase, decrease or cause no significant change to the antioxidant and carbohydrate-digestive enzyme inhibitory activities in the mushrooms.

Effect of in vitro digestion on the antioxidant and carbohydrate digestive enzyme inhibition activities in the cooked mushrooms

The antioxidant and carbohydrate-digestive enzyme inhibition activities of in vitro digested mushroom extracts were compared to their respective cooked counterparts (Table 4). In vitro digestion significantly increased the ferric reducing power of all cooked mushroom extracts by 80% to 1350% except for pressure-cooked *L. edodes* (– 75%). Among the digested samples, microwaved *A. polytricha* extract showed the highest increment in FRAP value. Similar trend was observed for the TEAC (45–2550%), DPPH (42–1400%) and TFC (437–3097%) in all cooked mushroom extracts after the digestion process. Pressure-cooked and microwaved *A. bisporus* extracts recorded the highest increment in TEAC and DPPH radical scavenging values respectively after the digestion. In vitro digestion also caused the highest increment in both TFC (3097%) and WPC (281%) of pressure-cooked *A. polytricha* extract.

In general, in vitro digestion improved the overall total antioxidant activity in the cooked mushroom extracts and this is in agreement with Chohan et al. (2012). The further breakdown of mushroom's cell wall matrix during the digestion process could facilitate the release of compounds with radical scavenging properties from the vacuole cells (Chandrasekara and Shahidi 2012). Besides, cooking has been shown to enhance the stability of antioxidant compounds under extreme pH condition through the formation of secondary structures (Kim et al. 2010). This study depicts the differential effect of digestion on WPC in the boiled and microwaved mushroom extracts. For instance, among the digested microwaved samples, significant increase in WPC was observed for *A. polytricha* (156%) and *L. edodes* (116%) but opposite trend was found in *A. bisporus* (– 48%) and *P. sajor-caju* (– 60%).

The effect of in vitro digestion on the carbohydrate-digestive enzymes inhibition activity varied according to the mushroom species and cooking methods applied. Digestion significantly decreased the anti- α -amylase activity in four microwaved mushroom extracts (*A. bisporus*, *A. polytricha*, *L. edodes* and *P. sajor-caju*) and three pressured-cooked mushroom extracts (*F. velutipes*, *L. edodes* and *P. sajor-caju*). Among these digested extracts, microwaved *A. polytricha* recorded the highest loss (– 99%) in activity. The decreased of anti- α -amylase activity in the cooked mushroom extracts after the intestinal digestion phase could be caused by the proteolytic action of trypsin on enzyme inhibitors (Frels and Rupnow 1985). In contrast, boiled *A. bisporus* (243%) and steamed *L. edodes* (753%) extracts showed significant improvement in anti- α -amylase activity after the digestion

Table 4 The total antioxidant activity and carbohydrate-digestive enzyme inhibition potential of cooked and in vitro digested mushroom samples

Cooked and digested mushrooms	Antioxidant activity		Phenolic content					Carbohydrate digestive enzyme inhibition potential					
	FRAP (mmol Fe ²⁺ /100 g)	Δ (%)	TEAC (mmol trolox/100 g)	Δ (%)	DPPH (mmol AA/100 g)	Δ (%)	WPC (mg GAE/100 g)	Δ (%)	TFC (mg QE/100 g)	Δ (%)	Anti-α-amylase (%) inhibition)	Anti-α-glucosidase (%) inhibition)	Δ (%)
<i>A. bisporus</i>													
b & d	0.32 ± 0.01 ^b	146	0.23 ± 0.06 ^b	109	0.28 ± 0.00 ^d	833	15.16 ± 1.30 ^a	107	476.44 ± 14.75 ^d	916	27.26 ± 6.74 ^a	243	28.40 ± 7.77
m & d	0.27 ± 0.01 ^c	145	0.39 ± 0.11 ^b	255	0.15 ± 0.00 ^d	1400	5.16 ± 1.06 ^a	− 48	384.00 ± 16.71 ^c	785	9.77 ± 3.91 ^b	− 89	24.70 ± 12.85
s & d	0.29 ± 0.02 ^b	163	0.16 ± 0.06 ^b	45	0.24 ± 0.02 ^b	500	10.96 ± 1.76		225.78 ± 33.77 ^a	588	53.15 ± 5.52		20.75 ± 1.21
p & d	0.20 ± 0.00 ^b	82	0.53 ± 0.07 ^b	2550	0.33 ± 0.02 ^b	725	9.32 ± 0.73		317.33 ± 6.67 ^c	437	5.82 ± 2.40		15.44 ± 1.76
<i>A. polytricha</i>													
b & d	0.14 ± 0.02 ^a	180	0.21 ± 0.03 ^c	320	0.32 ± 0.01 ^d	78	2.82 ± 1.20		479.56 ± 14.87 ^c	2918	44.36 ± 10.23		17.47 ± 3.00
m & d	0.29 ± 0.00 ^d	1350	0.44 ± 0.05 ^d	780	0.35 ± 0.02 ^b	119	11.76 ± 0.73 ^b	156	416.00 ± 10.07 ^d	1666	0.35 ± 0.07 ^a	− 99	76.88 ± 6.50 ^b
s & d	0.19 ± 0.01 ^b	111	0.20 ± 0.09 ^b	300	0.16 ± 0.03 ^a	700	7.82 ± 1.30		32.89 ± 35.51		24.52 ± 1.59 ^a	− 43	11.89 ± 0.48 ^b
P & d	0.05 ± 0.00 ^b	80	0.34 ± 0.07 ^a	750	0.36 ± 0.00 ^c	112	10.32 ± 0.47 ^c	281	536.44 ± 35.81 ^b	3097	69.75 ± 13.61		13.69 ± 0.95
<i>F. velutipes</i>													
b & d	0.05 ± 0.01		0.32 ± 0.03 ^c	433	0.32 ± 0.06 ^a	146	8.45 ± 1.30		279.56 ± 15.40 ^b	925	15.32 ± 3.33 ^a	− 55	19.99 ± 9.51
m & d	0.44 ± 0.05 ^b	633	0.36 ± 0.05 ^c	500	0.59 ± 0.05 ^b	321	8.35 ± 0.66		496.89 ± 10.78 ^d	1500	28.81 ± 6.41		23.94 ± 9.06
s & d	0.07 ± 0.03		0.29 ± 0.03 ^d	480	0.22 ± 0.01 ^a	47	7.22 ± 1.29		290.67 ± 36.97 ^b	447	24.92 ± 10.90		22.48 ± 1.56 ^a
p & d	0.12 ± 0.00 ^d	200	0.18 ± 0.06 ^b	260	0.31 ± 0.03 ^a	121	7.55 ± 0.39		260.44 ± 15.91 ^a	454	10.05 ± 3.69 ^d	− 88	38.87 ± 18.18
<i>L. edodes</i>													
b & d	0.30 ± 0.01 ^d	150	0.26 ± 0.04 ^c	225	0.29 ± 0.01 ^c	263	8.45 ± 2.06		451.11 ± 13.62 ^c	804	23.15 ± 4.55		51.60 ± 10.27
m & d	0.46 ± 0.01 ^d	254	0.48 ± 0.04 ^c	700	0.29 ± 0.01 ^b	222	17.58 ± 2.52 ^a	116	440.00 ± 31.44 ^b	842	10.97 ± 9.60 ^a	− 78	32.15 ± 4.68 ^b
s & d	0.28 ± 0.02 ^a	133	0.34 ± 0.08 ^b	467	0.29 ± 0.02 ^a	263	11.60 ± 2.14		498.22 ± 30.41 ^b	606	45.47 ± 7.14 ^a	753	11.46 ± 0.84
p & d	0.03 ± 0.00 ^d	− 75	0.37 ± 0.05 ^c	429	0.28 ± 0.01 ^a	87	9.85 ± 1.93		477.33 ± 2.67 ^a	591	11.77 ± 4.74 ^b	− 83	18.60 ± 2.40 ^a
<i>P. sajor-caju</i>													
b & d	0.20 ± 0.01 ^c	122	0.48 ± 0.03 ^b	1100	0.24 ± 0.02 ^b	100	3.99 ± 0.85 ^a	− 43	460.31 ± 12.16 ^c	706	52.76 ± 27.29		17.48 ± 4.59
m & d	0.16 ± 0.03 ^a	433	0.36 ± 0.05 ^c	620	0.29 ± 0.01 ^b	123	3.80 ± 1.40 ^a	− 60	789.33 ± 21.17 ^d	1738	8.82 ± 5.52 ^b	− 86	12.39 ± 0.55 ^a
s & d	0.12 ± 0.00 ^b	300	0.31 ± 0.07 ^b	520	0.17 ± 0.00 ^a	42	5.51 ± 1.24		48.89 ± 30.30		57.10 ± 28.85		4.05 ± 2.76 ^a
& d	0.10 ± 0.02		0.41 ± 0.03 ^d	583	0.36 ± 0.02 ^d	227	5.96 ± 1.32		405.33 ± 23.36 ^b	561	16.13 ± 2.34 ^b	76	10.91 ± 8.37

The values are presented as mean ± standard deviations (SD) of nine independent determinations; Δ —Significant variation when compared to the respective cooked sample; b & d—boiled and digested samples; m & d—microwaved and digested samples; p & d—pressure cooked and digested samples; s & d—steamed and digested samples; Values within the same column followed by superscript letters 'a' ($p < 0.05$), 'b' ($p < 0.01$), 'c' ($p < 0.005$) and 'd' ($p < 0.001$) were statistically different from the respective cooked species in Table 3

process. In vitro digestion also improved the anti- α -glucosidase activity in the microwaved (629%) and steamed (1251%) extracts of *A. polytricha*, steamed extract of *F. velutipes* (319%), microwaved (413%) and pressure-cooked (334%) extracts of *L. edodes*. Tadera et al. (2006) showed that gastrointestinal digestion could induce the release of aglycone phenolic acids with higher enzyme inhibition activity from the glycosides. However, the anti- α -glucosidase activities in the microwaved (-36%) and steamed (-83%) *P. sajor-caju* extracts were significantly decreased after the digestion process. Nevertheless, all cooked *A. bisporus* extracts were able to maintain their anti- α -glucosidase activities after the digestion.

Linear correlation and regression analysis

In this study, mushroom extracts with high ferric reducing power and radical scavenging activity were accompanied by high WPC and TFC, implying the contribution of phenolic compounds to the observed total antioxidant activity. This was supported by the significant positive correlation found between the total antioxidant activities (FRAP, TEAC, DPPH) and the phenolic contents (WPC & TFC) in the mushrooms (Table 5). Besides, the FRAP value was positively correlated with both TEAC ($r = 0.546$, $p < 0.005$) and DPPH radical scavenging values ($r = 0.472$, $p < 0.05$). It is pertinent to suggest the antioxidant compounds in the selected mushrooms were not only good reducing agents but also potent radical scavengers.

TFC in the mushroom extracts showed a weak but significant positive correlation with anti- α -glucosidase activity ($r = 0.323$, $p < 0.05$), possibly due to the effect of digestion which altered the flavonoid structural stability and rendered their binding to the enzymes. This explains the partial contribution of flavonoids to the anti- α -

glucosidase activity in the mushroom extracts. The galloyl groups of plant flavonoids have been shown as potent inhibitors for yeast α -glucosidase (Tadera et al. 2006). However, TFC was found to be inversely correlated with anti- α -amylase activity ($r = -0.363$, $p < 0.05$). Although WPC showed similar finding with both carbohydrate-digestive enzyme inhibition activities, they were not significant. The weak positive correlation found between the various antioxidant values (FRAP, $r = 0.385$, $p < 0.01$; TEAC, $r = 0.320$, $p < 0.05$; DPPH, $r = 0.299$, $p < 0.01$) and anti- α -glucosidase activity may suggest labile compounds other than antioxidants contributed to the carbohydrate-digestive enzymes inhibition activities in the mushroom extracts. For instance, the active form of β -glucan such as lentinan and pleuran found in *L. edodes* and *Pleurotus* spp. respectively have been shown to possess strong hypoglycaemic activity (Bach et al. 2017). This was further supported by the PCA result in this study that the anti- α -amylase activity was independent from the other activity groups (Fig. 1).

Principle component analysis (PCA)

PCA was used to provide an overview on the source of activity variation in the edible mushroom extracts subjected to different cooking methods and in vitro digestion process (Fig. 1). The two principle components shown in the biplot collectively represented the largest cumulative variance of 68.6% found in the data set. The first sub-component F1 accounted for 48.9% variation while the second sub-component F2 predicted another 19.7% variation. The x-axis (sub-component F1) separated the raw and cooked mushroom water extracts (negative area plot) from their digested counterparts (positive area plot), suggesting the 48.9% of variation corresponded to the antioxidant and carbohydrate-digestive enzymes inhibition activities in the

Table 5 Linear correlation of seven activity variables in the five edible mushroom samples

Variables	FRAP	WPC	TFC	TEAC	DPPH	Anti- α -amylase activity	Anti- α -glucosidase activity
FRAP	1.000						
WPC	0.372 ^a	1.000					
TFC	0.499 ^c	0.848 ^b	1.000				
TEAC	0.546 ^c	0.378	0.818 ^d	1.000			
DPPH	0.472 ^a	0.159	0.792 ^d	0.744 ^d	1.000		
Anti- α -amylase activity	-0.339^a	-0.059	-0.363^a	-0.442^b	-0.358^a	1.000	
Anti- α -glucosidase activity	0.385 ^b	0.241	0.323 ^a	0.320 ^a	0.299 ^b	0.184	1.000

The values denote the Pearson's correlation coefficient of 45 pairs of samples. The level of significance was expressed as $p < 0.05$ (^a); $p < 0.01$ (^b); $p < 0.005$ (^c) and $p < 0.001$ (^d)

Fig. 1 Principal component analysis of mushroom samples. *DPPH* 2,2-diphenyl-1-picryl-hydrazyl radical scavenging activity, *FRAP* ferric reducing antioxidant power, *TEAC* ferric reducing antioxidant power-ascorbic acid, *TFC* total flavonoid content, *WPC* water-soluble

phenolic content; b—boiled samples; d—in vitro digested samples; m—microwaved samples; p—pressure cooked samples; s—steamed samples

mushroom extracts was caused by the in vitro digestion process.

A clear separation of bioactivities in different mushroom extracts was observed in the second sub-component F2. The positive area on F2 axis was associated with WPC, ferric reducing power and anti- α -glucosidase activity while the negative area was linked with TFC, radical scavenging and anti- α -amylase parameters. These activity variables could be further separated into 3 activity groups with each of them located in different quadrants of the score plot. The first group consisted of WPC, FRAP and anti- α -glucosidase variables; the second group was made up of TFC and radical scavenging (DPPH and TEAC) variables while the last group was solely occupied by anti- α -amylase variable. The similar vector direction displayed by the variables in each of three activity groups indicated a strong relationship among them. For instance, the anti- α -amylase variable was closely associated with the different domestic cooking applied to the selected mushrooms. On the contrary, digestion was found to be closely associated with the antioxidant (WPC, TFC, FRAP, TEAC and DPPH) and anti- α -glucosidase activities in the mushroom extracts. The

above findings confirmed the influence of digestion and cooking process, at least partly, on the antioxidant and carbohydrate-digestive enzyme inhibition activities in the selected edible mushroom species.

Conclusion

In summary, the five edible mushrooms selected in this study could act as alternate food source for antioxidant and carbohydrate-digestive enzymes inhibitors. This study shows the potential use of heat treatment such as domestic cooking to potentiate the antioxidant and carbohydrate-digestive enzymes inhibition activities in the mushroom water extracts. To achieve the best antioxidant and carbohydrate-digestive enzymes inhibition values, *A. bisporus* and *F. velutipes* were best cooked with microwave while boiling was preferred for *A. polytricha*. Pressure cooking was shown as the preferred cooking method for *L. edodes* and *P. sajor-caju*. In vitro digestion could produce mushroom products with high anti- α -glucosidase but low anti- α -amylase activities for the control of postprandial

hyperglycaemia with minimal side effect. This study did not compare the bioavailability of bioactive compounds liberated from the mushroom extracts and absorbed through the intestinal borders. Further investigation with an in vivo model is required to provide a more comprehensive nutritional bioavailability profile across different mushroom species. Considering the vast consumption of mushrooms among the folks, the knowledge of heat treatment and in vitro digestion on the bioaccessibility of nutritional compounds in the mushrooms will benefit both the consumers and food manufacturers who aim for healthy food preparation, labelling and production.

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